REPORT

DETERMINATION OF THE PARTITION COEFFICIENT

(N-OCTANOL/WATER) OF

[redacted]

NOTOX Project 338614
NOTOX Substance 111834/B

STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

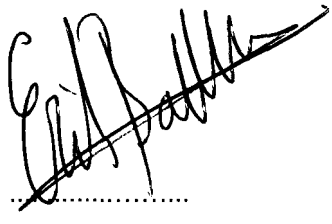
The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

CONFIDENTIALITY STATEMENT

This report contains the unpublished results of research sponsored by [redacted]. Reproduction, issue or disclosure to third parties in any form is not permitted without prior written authorisation from the sponsor.

Study Director

Dr.Ir. E. Baltussen



Date: 18 JUL 2002

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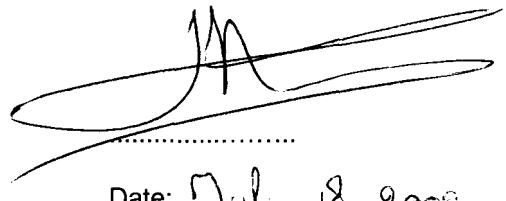

Date: July 18, 2002

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.
During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/AUDITS	REPORTING DATES
on-site inspection (s)	
18 February – 01 March 2002 (Process, physical chemistry)	04 March 2002
13 May – 31 May 2002 (Process, physical chemistry)	04 June 2002
protocol inspection (s)	
06 November 2001 (Study)	06 November 2001
report audit (s)	
18 June 2002 (Study)	18 June 2002

Head of Quality Assurance
C.J. Mitchell B.Sc.

Date: 22-7-02.

SUMMARY

The determination of the partition coefficient (n-octanol/water) of [redacted] was based on the following guidelines:

OECD Guidelines for Testing of Chemicals, Guideline No. 117: "Partition coefficient (n-octanol/ water) High Performance Liquid Chromatography (HPLC) method" (adopted March 30, 1989).

EEC directive 92/69 EEC, Part A, Methods for the determination of physico-chemical properties, A.8: "Partition coefficient", EEC Publication no. L383, December 1992.

Since [redacted] is a mixture of compounds, the HPLC method was chosen for the determination of the partition coefficient (n-octanol/water). The Rekker calculation method was also performed.

Rekker calculation method

From the structural formula of [redacted] the log P_{ow} was calculated to be –0.95, 0.22, 0.75 and 2.20 (P_{ow} = 0.11, 1.66, 5.62 and 158).

HPLC method

The chromatograms of the preliminary test showed a single peak and no test substance peaks with a retention time larger than 2,4-DDT.

Using 50/50 (v/v) methanol/Milli-Q water as the mobile phase, a 125 mm LiChrospher 100 RP-18 column (Merck) and a spectrophotometric detector set to read the absorbance at 210 nm, one large and five small test substance peaks were observed in the HPLC chromatograms of [redacted] The large peak probably derives from the major component in [redacted] nd the small peaks probably derive from minor components in [redacted] The corresponding P_{ow} and log P_{ow} values are:

Major component: $P_{ow} = 3.60 \times 10^1$ (log P_{ow} = 1.6)

Minor component 1: P_{ow} = 1.84 (log P_{ow} = 0.27)
Minor component 2: P_{ow} = 7.06 (log P_{ow} = 0.85)
Minor component 3: $P_{ow} = 1.36 \times 10^2$ (log P_{ow} = 2.1)
Minor component 4: $P_{ow} = 1.65 \times 10^3$ (log P_{ow} = 3.2)
Minor component 5: $P_{ow} = 2.47 \times 10^3$ (log P_{ow} = 3.4)

The temperature of the mobile phase was 24.5 ± 1.0°C during the test.

Conclusion

The results of the Calculation method and the HPLC method are not in agreement. Since the HPLC method is a more accurate method than the Calculation method, the result of the HPLC method is reported as the partition coefficient (n-octanol/water), P_{ow}, of Trigonox R-938.

The P_{ow} value for the major component in [redacted] is 3.60×10^1 (log P_{ow} = 1.6) at 24.5 ± 1.0°C.

The P_{ow} values for five minor components in [redacted] are 1.84, 7.06, 1.36×10^2, 1.65×10^3 and 2.47×10^3 (log P_{ow} values of 0.27, 0.85, 2.1, 3.2 and 3.4) at 24.5 ± 1.0°C.

In this test, the major component has a log P_{ow} of 1.6, which is in accordance with literature data indicating that the main component by weight, dimethylphthalate, has a log P_{ow} of 1.6 (Ellington and Floyd, USEPA/600/S-96/001, Sept. 1996).

According to literature, the component 3-methyl-2-butanone has a log P_{ow} of 0.84 (Hansch, C et al, 1995).

PREFACE

Sponsor	[redacted]
Study Monitor	Dr. C.L.J. Braun SHERA, Regulatory Affairs
Testing Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Dr.Ir. E. Baltussen
Study plan	Start: 28 November 2001 Completed: 29 May 2002

TEST SUBSTANCE

Identification	[redacted]
Chemical name	[redacted]
CAS RN	[redacted]
Description	Clear colourless liquid
Batch	1510-14
Purity	See Certificate of Analysis
Test substance storage	In refrigerator in the dark
Stability under storage conditions	Stable
Expiry date	01 January 2003

The sponsor is responsible for all test substance data unless determined by NOTOX.

Note: **Don't heat up the test substance above 50°C**

PURPOSE AND PRINCIPLE

The purpose of the study was to determine the partition coefficient of the test substance between n-octanol and water.

The partition coefficient (n-octanol/water) (P_{ow}) is defined as the ratio of the equilibrium concentrations in a two-phase system consisting of n-octanol and water.

Four different methods are available for the determination of the partition coefficient.
The principle of each method is described below:

1. Flask-shaking method
   Dissolved test substance is mixed thoroughly with n-octanol and water. After phase separation, the concentration of the test substance in both phases is determined by a suitable analytical method.

2. HPLC-method
   A solution of the test substance is injected onto a reversed-phase HPLC-column. The log P_{ow}-value is calculated from the retention time of the test substance.

3. Estimation method
   The solubilities of the test substance in n-octanol and in water are determined. The quotient of the n-octanol solubility and the water solubility is a rough estimation of the partition coefficient.

4. Calculation from the structural formula (Rekker calculation method)
   Calculation methods are based on the theoretical fragmentation of the molecule into suitable substructures for which reliable log P_{ow} increments are known. The log P_{ow} is obtained by summing the fragment values and the correction terms for intramolecular interactions.
   Lists of fragment constants and correction terms are available.

Since [redacted] is a mixture of compounds, the HPLC method was chosen for the determination of the partition coefficient (n-octanol/water). The Rekker calculation method was also performed.

[redacted]

The study procedure described in this report was based on the following guidelines:

Organization for Economic Co-operation and Development (OECD), OECD Guideline for Testing of Chemicals, Guideline No. 117: "Partition coefficient (n-octanol/water) High Performance Liquid Chromatography (HPLC) method" (adopted March 30, 1989).

European Economic Community (EEC), EEC directive 92/69 EEC, Part A, Methods for the determination of physico-chemical properties, A.8: "Partition coefficient", EEC Publication no. L383, December 1992.

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years:
protocol, report, test substance reference sample and raw data.

SYSTEM DESCRIPTION/CALCULATION METHOD

For the calculation of the partition coefficient from the structural formula of [redacted] the following hardware and software were used:

Hardware	Personal computer, Intel Pentium 166 MMX, 32 MB DRAM, operating system Windows NT 4.0
Software	PrologP version 5.1 (module in PALLAS version 2.1), an expert system for the calculation of log P_{ow} using the Rekker method. The program was purchased from CompuDrug Chemistry Ltd., Budapest, Hungary.

TEST SYSTEM AND RATIONALE/HPLC METHOD

A High Performance Liquid Chromatograph with a spectrophotometric detector set to read the absorbance at 210 nm was used. The stationary phase was silica bonded C18. The mobile phase was 75/25 (v/v) methanol/Milli-Q water for a preliminary test and
50/50 (v/v) methanol/Milli-Q water for the main study. See also the section 'HPLC conditions/HPLC method'.

The test system is recognised by the international guidelines (OECD, EEC).

REAGENTS/HPLC METHOD

Formamide	P.a., Merck, Darmstadt, Germany
2,4-DDT	98%, Dr. Ehrenstorfer, Augsburg, Germany
Methanol	HPLC-grade, Labscan, Dublin, Ireland
Milli-Q water	Tap water purified by reversed osmosis and subsequently passed over activated carbon and ion-exchange cartridges; Millipore, Bedford, MA, USA

REFERENCE SUBSTANCES/HPLC METHOD

Comparing the retention time(s) of a test substance with those from reference substances with known log P_{ow} values can yield log P_{ow} value(s) for the test substance. Six chemicals for which log P_{ow} has been reported were used to prepare a mixture of reference substances.

Reference substance	Log P_{ow}[1]
- ethylmethylketone (> 99%, Acros, Geel, Belgium)	0.3
- nitrobenzene (>99.5%, Fluka Chemie, Buchs, Switzerland)	1.9
- toluene (p.a., Merck)	2.7
- bromobenzene (99%, Sigma-Aldrich, Steinheim, Germany)	3.0
- 1,4-dichlorobenzene (f.s., Merck)	3.4
- biphenyl (99%, GC, Sigma-Aldrich)	4.0

[1] log P_{ow} values according to the OECD guideline.

PERFORMANCE OF THE TEST/CALCULATION METHOD

The determination was performed using the Rekker calculation method, using a computer program and associated data base of fi and interaction factors. The operation of the computer program is described in the ProLogP user's manual.

Theory
Calculation methods are based on the theoretical fragmentation of the molecule into suitable substructures for which reliable log P_{ow} increments are known. The log P_{ow} is obtained by summing the fragment values and the correction terms for intramolecular interactions.
Lists of fragment constants and correction terms are available.

In general, the reliability of calculation methods decreases as the complexity of the compound under study increases. In the case of simple molecules of low molecular weight and with one or two functional groups, a deviation of 0.1 to 0.3 log P_{ow} units between the results of the different fragmentation methods and the measured value can be expected. The margin of error will depend on the reliability of the fragment constants used, the ability to recognise intramolecular interactions (e.g. hydrogen bonds) and the correct use of correction terms. In the case of ionising compounds the charge and degree of ionisation must be taken into consideration.

Rekker calculation method
Using the Rekker method the log P_{ow} value is calculated as:

$$\log P_{ow} = \sum a_i f_i + \sum (\text{interaction terms})$$

where a_i is the number at which a given fragment is present in the molecule and f_i is the log P_{ow} increment of the fragment. The interaction terms can be expressed as an integral multiple of one single constant C_m (so-called "magic constant"). The fragment constants f_i and C_m have been determined from an extended set of about 1000 log P_{ow} values in the n-octanol/water system. However a number of modifications were introduced into this collection in order to make it more applicable.

PERFORMANCE OF THE TEST/HPLC METHOD

Preparation of the solutions (preliminary test)

A 0.96 g/l solution of 2,4-DDT in methanol was diluted by a factor 100 with mobile phase (i.e. 75/25 (v/v) methanol/Milli-Q water).

A 10994 mg/l stock solution of [redacted] was prepared in methanol. The test solution was prepared by diluting the stock solution by a factor 10 with mobile phase. Methanol diluted by a factor 10 with mobile phase was used as the test substance blank solution.

Preparation of the solutions (main study)

Solutions of the reference substances (except for ethylmethylketone) in methanol, at concentrations between 1.2 and 1.6 g/l, were used. A mixture of the reference substances was prepared by adding 50 µl of each reference substance solution and 1.0 ml ethylmethylketone to a 5.0 ml volumetric flask. A blank solution was prepared by adding 250 µl of methanol to a 5.0 ml volumetric flask. These flasks were subsequently filled up to the mark with mobile phase (i.e. 50/50 methanol/Milli-Q water).

A 1.9 g/l solution of formamide in methanol was diluted by a factor 10 with mobile phase. This solution was used for the determination of t_0 (retention time of the unretarded component). As formamide blank solution, methanol diluted by a factor 10 with mobile phase was used.

A 10994 mg/l stock solution of [redacted] was prepared in methanol. The test solution was prepared by diluting the stock solution by a factor 10 with mobile phase. Methanol diluted by a factor 10 with mobile phase was used as the test substance blank solution.

HPLC injections (main study)

The solutions were injected in the HPLC system in the following sequence: the mixture of reference substances, the reference substances blank solution, the formamide solution, the formamide blank solution, the [redacted]est solution (in duplicate), the test substance blank solution, the mixture of reference substances and the formamide solution. The chromatograms of the formamide solution and the corresponding blank were recorded for 5 minutes. All other chromatograms were recorded for 110 minutes.

The temperature of the mobile phase (24.5 ± 1.0°C) was recorded intermittently during the measurements.

HPLC CONDITIONS/HPLC METHOD

Column	LiChrospher 100 RP-18; 125 * 4 (I.D.) mm; d_p= 5 µm (Merck, Germany)
Mobile phase (preliminary test)	75/25 (v/v) methanol/Milli-Q water
Mobile phase (main study)	50/50 (v/v) methanol/Milli-Q water
Flow	1 ml/min
Wavelength of detection	210 nm
Injection volume	10 µl

DATA HANDLING/HPLC METHOD

The capacity factor (k') for a substance is calculated from the retention of the substance concerned (t_r) and the unretarded component (t_0): $k' = (t_r - t_0)/t_0$

The log k' values obtained for the references substances were plotted against the known log P_{ow} values. A linear regression program was used to calculate the regression line:
$\log k' = a * \log P_{ow} + b$.
Linear regression analysis was performed using the least squares method. The coefficient of correlation was also calculated.

The log P_{ow} value for each component in the test substance was calculated by substituting its log k' in the regression line.

RESULTS/CALCULATION METHOD

From the structural formula of Trigonox R-938, the log P_{ow} was calculated to be –0.95, 0.22, 0.75 and 2.20 (P_{ow} = 0.11, 1.66, 5.62 and 158), using the Rekker calculation method.

RESULTS/HPLC METHOD

The chromatograms of the preliminary test showed a single peak and no test substance peaks with a retention time larger than 2,4-DDT.

Using 50/50 (v/v) methanol/Milli-Q water as the mobile phase, a 100 RP-18 column and a spectrophotometric detector set to read the absorbance at 210 nm, one large and five small test substance peaks were observed in the chromatograms of the 1099.4 mg/l [redacted] test solution which were recorded for 110 minutes. The temperature of the mobile phase was 24.5 ± 1.0°C during the test. It was assumed that the large peak derives from the major component of Trigonox R-938 whereas the small peaks derive from minor components in [redacted]

HPLC chromatograms of the [redacted] test solution, the test substance blank solution, the mixture of reference substances, the reference substances blank solution, the formamide solution and formamide blank solution are shown in Figures 1 to 6.

The t_0 (the retention time of the unretarded component) was determined to be 1.104 minutes as a mean value of both measurements (i.e. 1.104 and 1.104 minutes).

The mean values of the retention times, k' values, log k' values, log P_{ow} and P_{ow} values are summarised in Table 1.

The calculations were performed using not-rounded values. Therefore, some differences might be observed when re-calculating the k' values, the log k' values, the log P_{ow} values and the P_{ow} values in the table below.

Table 1 Results of the HPLC method.

Substance	t_r [1]	k'	log k'	log P_{ow}	P_{ow}
Reference substance					
Ethylmethylketone	1.68	0.517	-0.287	0.3	
Nitrobenzene	5.88	4.33	0.636	1.9	
Toluene	17.2	14.6	1.16	2.7	
Bromobenzene	22.1	19.0	1.28	3.0	
1,4-dichlorobenzene	37.4	32.8	1.52	3.4	
Biphenyl	81.0	72.3	1.86	4.0	
Test substance					
[redacted]					

[1] Mean value of the retention times of two chromatograms.

* Interpolated from the regression line: log k' = 0.582 * log P_{ow} - 0.456 (r=0.9995, n = 6)

** Extrapolated from the regression line: log k' = 0.582 * log P_{ow} - 0.456 (r=0.9995, n = 6)

CONCLUSION

The results of the Calculation method and the HPLC method are not in agreement. Since the HPLC method is a more accurate method than the Calculation method, the result of the HPLC method is reported as the partition coefficient (n-octanol/water), P_{ow}, of [redacted]

The P_{ow} value for the major component in [redacted] is 3.60×10^1 (log P_{ow} = 1.6) at 24.5 ± 1.0°C.

The P_{ow} values for five minor components in [redacted] are 1.84, 7.06, 1.36×10^2, 1.65×10^3 and 2.47×10^3 (log P_{ow} values of 0.27, 0.85, 2.1, 3.2 and 3.4) at 24.5 ± 1.0°C.

In this test, the major component has a log P_{ow} of 1.6, which is in accordance with literature data indicating that the main component by weight, dimethylphthalate, has a log P_{ow} of 1.6 (Ellington and Floyd, USEPA/600/S-96/001, Sept. 1996).

According to literature, the component 3-methyl-2-butanone has a log P_{ow} of 0.84 (Hansch, C et al, 1995).

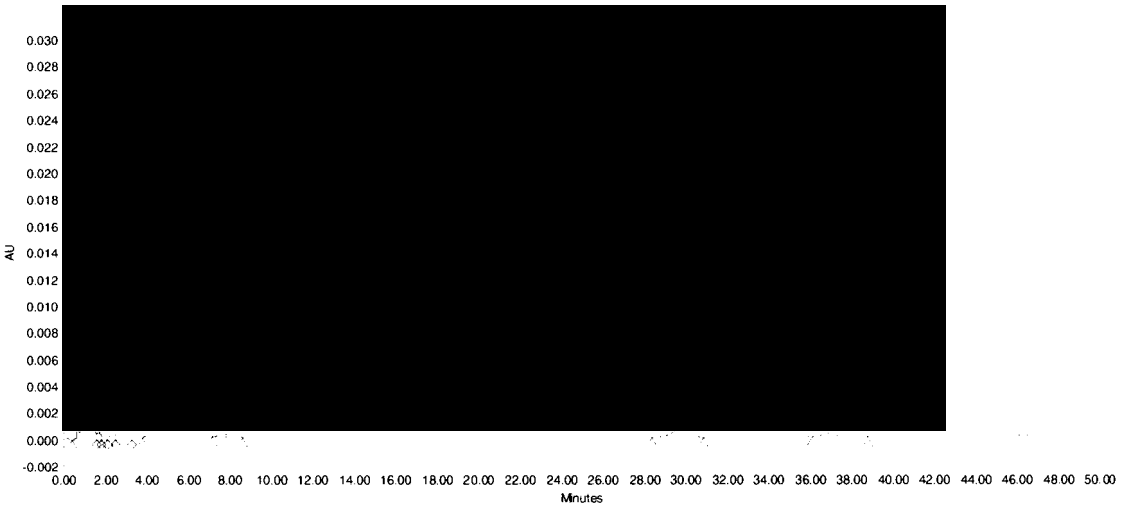

Figure 1 HPLC chromatogram of the 1099.4 mg/l [redacted]est solution [res.id.1411].

Note: The peaks at 1.09 and 2.59 minutes were also observed in the chromatograms of the blank solution (see Figure 2, peaks at [redacted]). Therefore it was assumed that these peaks did not derive from the test substance. The chromatogram was recorded for 110 minutes. No peaks were observed between 50 and 110 minutes.

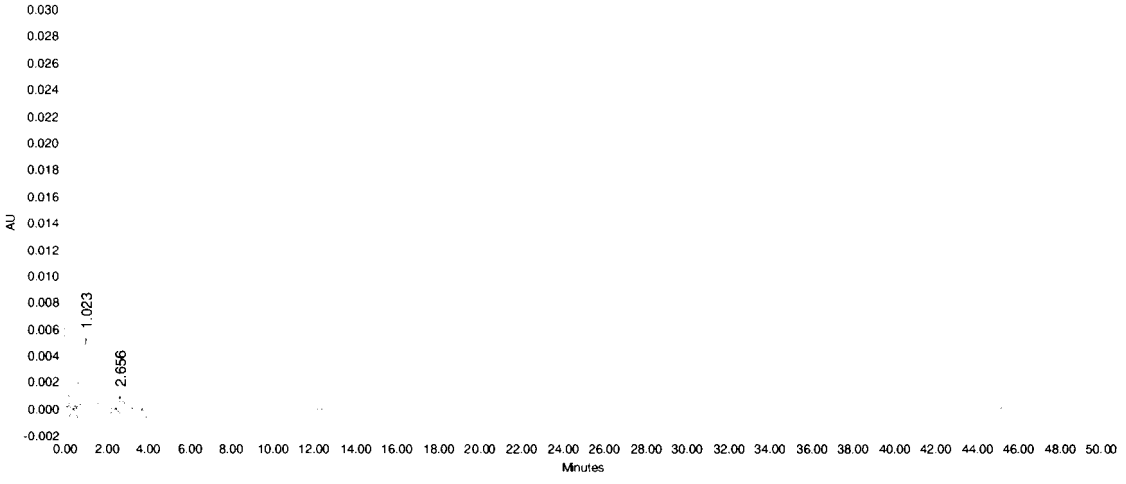

Figure 2 HPLC chromatogram of the test substance blank solution [res.id. 1410].

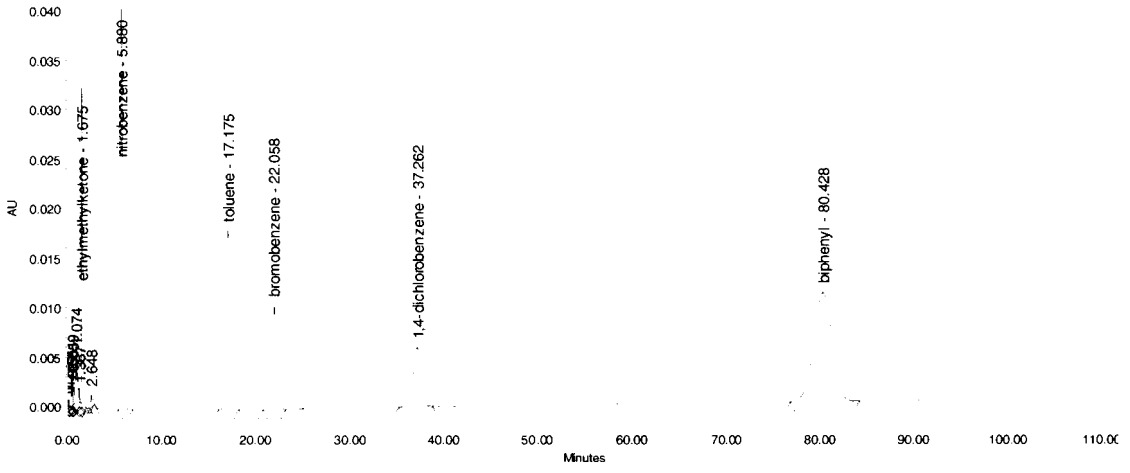

Figure 3 HPLC chromatogram of the mixture of reference substances [res.id. 1331].

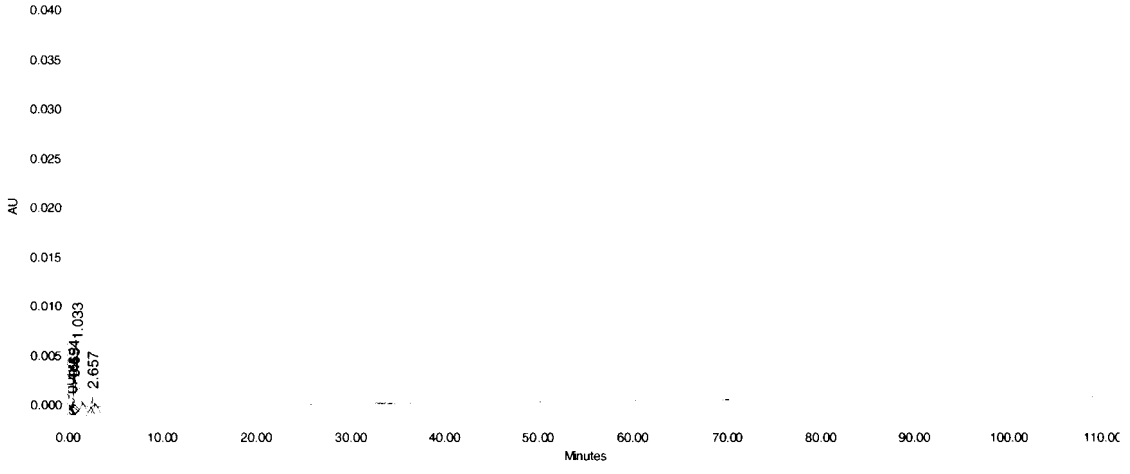

Figure 4 HPLC chromatogram of the reference substances blank solution [res.id. 1329].

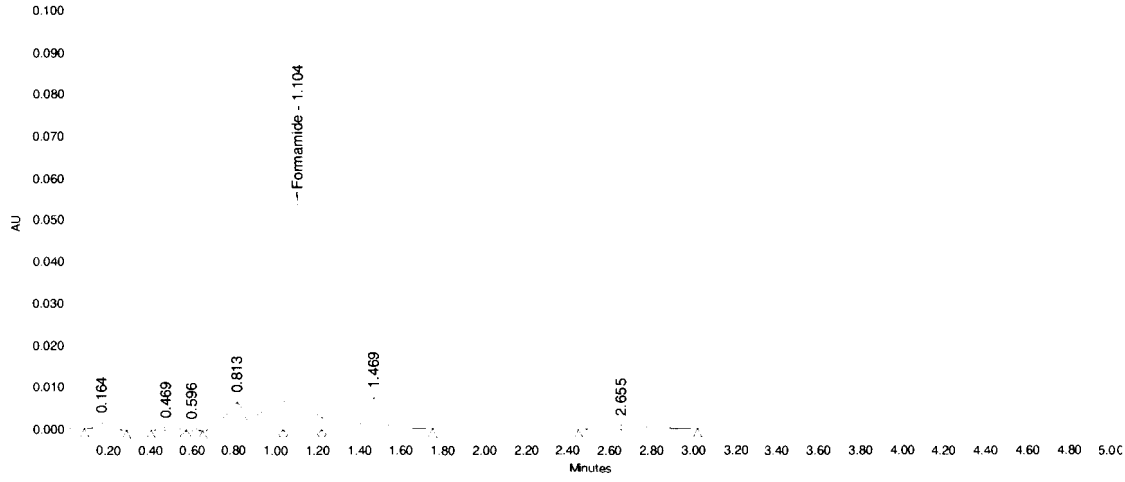

Figure 5 HPLC chromatogram of the formamide solution [res.id. 1308].

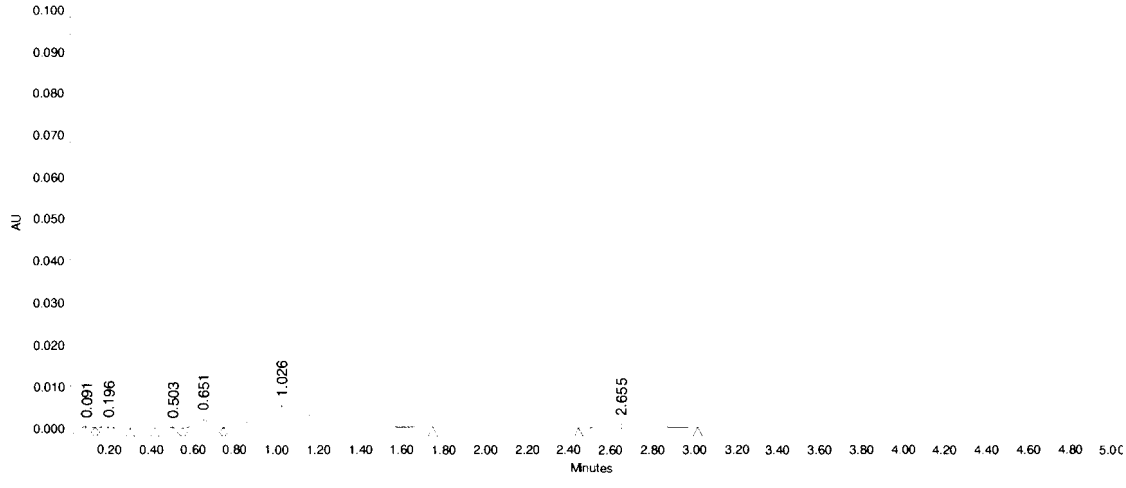

Figure 6 HPLC chromatogram of the formamide blank solution [res.id. 1306].

CERTIFICATE OF ANALYSIS

[redacted]

Certificate of Analysis [redacted]

TNA-2001007
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ICS-331

Product name : [redacted]

Chemical name : [redacted]

Batch number : **1510-14**

Test results:

Method	Analysis of	Unit	Result *[1]
Jo/72.11, Jo/95.2	Peroxidic compounds (sum) ***See page 2 for a specification***	% m/m	28.6 (± 1.5)
J20010792	[redacted]	% m/m	67.0 (± 1.0)
J20010792	I[redacted]	% m/m	2.0 (± 0.3)
Amp/88.9	Water	% m/m	2.6 (± 0.3)
J20010792	Unidentified impurities	% m/m	0.5 (± 0.2)

*[1] bracketed values are estimated 95% confidence intervals

File code : **TNA-2001007**

Analytical documentation : 20010792

[redacted]

[redacted]

Certificate of Analysis [redacted]

[redacted] batch 1510-14: specification of the peroxidic compounds

structure	% m/m
[redacted]	

[redacted]